2002

Assembling and Characterizing a Comprehensive Echinacea Germplasm Collection

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Reprinted from: Trends in new crops and new uses. 2002. J. Janick
and A. Whipkey (eds.). ASHS Press, Alexandria, VA.

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Mark P. Widrlechner and Kathleen A. McKeown

INTRODUCTION

During the 1990s, the popularity of the genus *Echinacea* Moench (Asteraceae) as a dietary supplement in the United States increased markedly, as the general public learned of its possible efficacy in fighting colds and other illnesses (Bauer and Wagner 1991; Li 1998). Plant and medical scientists responded to this phenomenon by increasing their efforts to understand the biology, cultivation, and pharmacology of these plants. Unfortunately, very few well-documented living collections of *Echinacea* were readily available to support that research. Well-documented germplasm collections could also be used to broaden the genetic base of ornamental *Echinacea* cultivars, which are widely cultivated as attractive landscape perennials.

METHODOLOGY

In response, during the mid-1990s, the North Central Regional Plant Introduction Station (NCRPIS) began assembling germplasm collections from wild *Echinacea* populations from throughout its native range in the United States and Canada. These efforts were given a great boost in 1997, when the United States Department of Agriculture, Agricultural Research Service sponsored the location and collection of seed samples representing the diversity of all known *Echinacea* taxa (McKeown 1999a). By the end of the decade, the NCRPIS had acquired samples of more than 130 different wild populations, including all recognized taxa (Table 1, Fig. 1). In 1999, the NCRPIS began a project to produce sufficient quantities of seeds from these

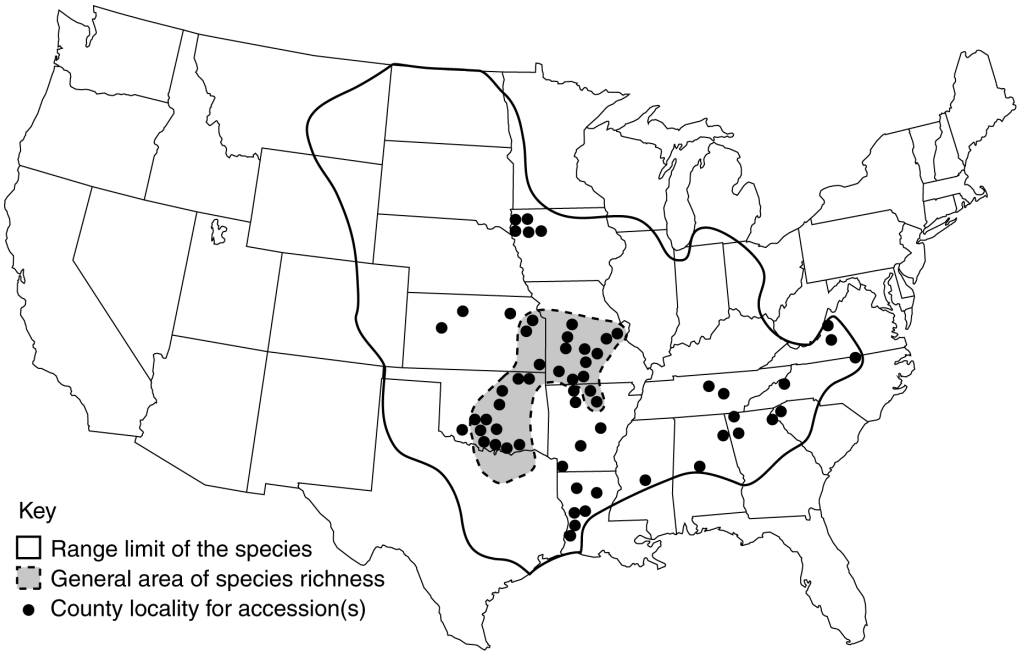

Fig. 1. Map of the natural range of *Echinacea* in the United States and collection sites (McKeown 1999b).

*Journal Paper No. J-19678 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 1018, and supported by Hatch Act and State of Iowa. The authors wish to thank Paul Scott, Loren Stephens, and Michael Purugganan for their valuable critiques of this paper. The junior author gratefully acknowledges the Charles W. Stuber Endowment Fund, the U.S. Department of Education Graduate Fellowship in an Area of National Need (Biotechnology), and the Department of Genetics at North Carolina State University for generous support.

Table 1. *Echinacea* germplasm conserved at the North Central Regional Plant Introduction Station.

Taxon	No. accessions
E. angustifolia DC.	17
E. angustifolia var. *angustifolia*	22
E. angustifolia var. *strigosa* McGregor	2
E. atrorubens Nutt.	5
E. hybrid	3
E. laevigata (F.E. Boynton & Beadle) S.F. Blake	10
E. pallida (Nutt.) Nutt.	39
E. paradoxa (Norton) Britton var. *neglecta* McGregor	4
E. paradoxa var. *paradoxa*	5
E. purpurea (L.) Moench	13
E. sanguinea Nutt.	9
E. simulata McGregor	8
E. tennesseensis (Beadle) Small	4

populations, both to conserve the populations (which are often threatened in nature by commercial exploitation) and to make seeds freely available to the research community. The regeneration project cultivates individual populations within screened cages (Fig. 2) with honeybees as pollinators to produce control-pollinated seeds (Widrlechner et al. 1997).

RESULTS, DISCUSSION, AND FUTURE RESEARCH

In 2000, sufficient numbers of control-pollinated seeds were produced from more than 80 populations to allow their distribution, with additional harvests made during the late summer and autumn of 2001. During the course of the regeneration project, notes and measurements were collected on a wide range of morphological descriptors, and taxonomic identities were verified. These characterization data are being prepared for inclusion in the Germplasm Resources Information Network (GRIN) database, which is accessible on the Internet at <www.ars-grin.gov/npgs>. In late September, 2001, at the end of three years in the field and a final seed harvest, roots were dug, dried, and shipped for chemical analysis of bioactive compounds (caffeic acid derivatives and alkamides) by James Simon's laboratory at Rutgers University.

Beyond conducting morphological and biochemical characterization, there is a need to understand the genetic basis of such variation. Which genes are responsible for the unusual biochemistry of this genus? How are they shared among the taxa? Can they be transferred to existing crops, such as sunflower, to confer natural insect or fungal resistance? How can *Echinacea* best be domesticated, both as an ornamental and as a dietary supplement to replace wild harvesting?

To begin to answer these questions a preliminary evaluation of genetic diversity within both the genus and selected accessions is underway. Phylogeographic analysis, a novel approach to plant germplasm evaluation of genetic diversity, will be the primary methodology utilized. The power of phylogeographic analysis lies in consideration of the

Fig. 2. Control-pollinated seed multiplication of *E. pallida* in a screen cage (image by A.P. Ovrom).

spatial distribution of genealogical lineages inferred from nucleotide sequence variation, which is an absolute measure of genetic diversity. *Echinacea* taxa are morphologically and chemically similar and exhibit broad phenotypic plasticity, particularly under varying photoperiod regimens (K. McKeown, unpubl. obs.) A sequence-based analysis of diversity among *Echinacea* taxa is an essential counterpart to phenotypic-based evaluations.

Analyses of nucleotide sequence variation are conducted on the same region in a given locus for all taxa considered. In particular, a gene region that is "selectively neutral" in molecular evolutionary terms is required. In this context, "selectively neutral" indicates a region in the genome where mutations (leading to nucleotide substitutions, insertions, or deletions) have no effect on the fitness of the organism. For example, introns are generally neutral, and a number of statistical tests on the sequence data are utilized to demonstrate this neutrality (Li 1997). Nonrandom geographic patterns of genetic variation would suggest significant genetic divergence. The potential utility of phylogeography to plant germplasm conservation is apparent when the pattern of sequence variation is considered in association with the geographic spatial distribution of the taxa in question (Avise 2000).

Glyceraldehyde 3-phosphate dehydrogenase (G3PDH) is an enzyme in the glycolytic pathway, encoded by a nuclear gene in plants. Sequence variation at the *G3pdh* locus has recently been used to unravel the geographic origin of domesticated cassava (Olsen and Schaal 1999). A sample of 40 *Echinacea* populations was chosen for analysis by selecting populations collected nearest to the intersection of whole-number longitudes and latitudes between 78°–99° West and 30°–40° North. Preliminary nucleotide sequence data of the noncoding region of the *G3pdh* locus across these populations reveal useful variation, and geographic mapping of haplotypes is underway. This will be the first phylogeographic analysis of a medicinal plant based on the nuclear genome, the genome which characteristically has the greatest degree of nucleotide polymorphism in plants.

An Amplified Fragment Length Polymorphism (AFLP) analysis (Vos et al. 1995) of *Echinacea* populations will also be conducted. This will generate a large set of markers, the frequency and identity of which will be used for calculating genetic distances, quantifying overall levels of diversity and fine-scale genetic mapping. These genome-wide markers will also provide an important comparison for findings based on the analysis of *G3pdh*.

REFERENCES

Avise, J.C. 2000. Phylogeography: The history and formation of species. Harvard Univ. Press, Cambridge.

Bauer, R. and H. Wagner. 1991. *Echinacea* species as potential immunostimulatory drugs. Econ. Med. Plant Res. 5:253–321.

Li, T. 1998. *Echinacea*: Cultivation and medicinal value. HortTechnology 8:122–129.

Li, W.H. 1997. Molecular evolution. Sinauer Associates, Inc., Sunderland, MA.

McKeown, K.A. 1999a. Echinacea gives U.S. the opportunity to put conservation policies into practice. Diversity 15(3):17–19.

McKeown, K.A. 1999b. A review of the taxonomy of the genus *Echinacea*. p. 482–489. In: J. Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

Olsen, K.M. and B.A. Schaal. 1999. Evidence on the origin of cassava: Phylogeography of *Manihot esculenta*. Proc. Nat. Acad. Sci. (USA) 96:5586–5591.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.

Widrlechner, M.P., C.A. Abel, and R.L. Wilson. 1997. Ornamental seed production in field cages with insect pollination. Combined Proc. Int. Plant Prop. Soc. 46:512–516.